Pre-Treatment Procedures

- Animal health procedure: all animals received a clinical examination for ill-health on arrival and a veterinary clinical examination during the acclimatization period.
- Acclimatization period: at least 3 weeks between animal arrival and start of treatment.

Experimental Design

- Allocation to treatment groups was performed during the acclimatization period using a random allocation procedure based on body weight classes.
- Animals were assigned to the treatment groups shown in Table 1. The dose levels administered were shown in Table 2.

Administration of the Test/Control Articles

Group 1 and 2 Animals

- Method of administration: injection in the left inguinal lymph node. Animals were lightly anaesthetized before each administration by an intramuscular injection of ketmine hydrochloride (Imalgene® 500 - Merial, Lyon, France). The same lymph node was injected on each occasion (left side). Each injection was followed by a local disinfection with iodine (Vétédine® - Vétoquinol, Lure, France).

Group 3

- Route: subcutaneous.
- Method of administration: bolus injection using a sterile syringe and needle introduced subcutaneously. Four injection sites were used followed by a local disinfection with iodine (Vétédine® - Vétoquinol, Lure, France). Animals were also lightly anaesthetized before each administration by an intramuscular injection of ketamine hydrochloride (Imalgene® 500 - Merial, Lyon, France) in order to be under the same conditions as groups 1 and 2 animals.

Four injection sites in the dorsal cervical/interscapular regions were used as shown in Table 3.

- **ELISPOT Analysis**

An ELISPOT assay was used in order to assess the cell mediated immune response generated in the monkeys in the various treatment groups. In particular, an ELISPOT IFNγ assay was used in order to measure IFNγ production from T lymphocytes obtained from the monkeys in response to gp100 antigens.

Materials and Methods

Plates: MILLIPORE Multiscreen HA plate / MAHA S45.10 (96 wells).

Capture antibodies: MABTECH monoclonal anti-IFNγ antibodies/G-Z4 1 mg/mL.

Detection antibodies: MABTECH monoclonal anti-IFNγ antibodies/7-B6-1-biotin 1 mg/mL.

Enzyme: SIGMA, Extravidin-PA conjuate/E2636

Substrate: BIORAD, NBT/BCIP - Alkaline phosphatase conjugate substrate kit/ref: 170-64 32.

Coating

Place 100 µL per well of capture antibodies at 1 µg/mL diluted at 1/1000 in carbonate bicarbonate buffer 0.1M pH 9.6 into the multiwell plate. Incubate overnight at 4°C. Wash 4 times in 1X PBS.

Saturation

Place 200 µL per well of RPMI supplemented with 10% FCS, non essential amino acids, pyruvate, Hepes buffer and Peni-Strepto. Incubate 2 hours at 37°C.

Test

Cells from the immunized animals are tested against (a) medium alone; (b) pooled peptides at a concentration of 1 mg/mL; and (c) a non specific

stimulus (PMA-Iono). The pooled peptides used in this Example to stimulate IFN-γ production were derived from gp100 and are illustrated in Tables 4 to 7. The final volume of each sample is 200 µL. Incubate 20 hours at 37°C.

Wash 4 times in 1X PBS and 0.05% Tween 20.

Detection

Place 100 µL per well of detection antibodies at 1 µg/mL diluted in 1/1000 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 2 hours at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

Reaction

Place 100 µL per well of Extravidin-PA conjugate diluted 1/6000 in 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 45 minutes at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

Substrate Addition

Place 100 µL per well of substrate previously prepared. For example, for 1 plate, prepare: 9.6 mL of distilled water, 0.4 mL of 25X buffer, 0.1 mL of solution A (NBT) and 0.1 mL of solution B (BCIP). Incubate 30-45 minutes at room temperature. Wash in distilled water. Dry and transfer to a plastic film. The number of spots are counted using a Zeiss image analyzer. Each spot corresponds to an individual IFN-γ secreting T cell.

Results

The animals that tested positive on the ELISPOT analysis are shown in Figures 1-4. Overall, the results demonstrate that of the animals tested, 2 out of 2 (i.e. 100%) of the animals that received the intranodal administration of the gp100 antigen, and 2 out of 4 (i.e. 50%) of the animals that received the subcutaneous administration of the gp100 antigen had a positive cell mediated immune response.

ELISA Analysis

The ELISA was performed utilizing standard methodology known in the art. Briefly, the human gp100 ("hgp100"; produced in Baculovirus) was diluted in coating buffer (carbonate-bicarbonate, pH9.6) and added to 96 wells at 0.5ug/well. Plates were placed at 4°C overnight. Plates were then washed and blocking buffer (phosphate buffered saline/0.5% Tween 20/1.0% BSA, pH7.2) was added for 2 hours at 37°C. The plates were then washed and the sera was diluted in dilution buffer (phosphate buffered saline/0.5 % Tween 20/ 0.1 BSA, pH7.2). For this study, monkey sera was diluted to 1:800 and "7" serial 3 fold dilutions were done for each sample tested. The human sera controls were diluted to 1:50 in dilution buffer and "7" serial 2 fold dilutions were performed. Each dilution was done in duplicate. The plates were incubated a further 2 hours at 37°C. The plates were washed and the horse radish peroxidase (HRP)-conjugated anti-human secondary antibody (anti-human Ig whole antibody from sheep (Amersham Life Science, NA933)) diluted 1:100 in dilution buffer was added to the wells and incubated for 1 hour at 37°C. The plates were washed and OPD (o-phenylenediamine dihydrochloride) substrate with H_2O_2 in substrate buffer (50mM phosphate/25mM citrate, pH 7.2) was added to the wells. For a kinetics ELISA, the plate was read repeatedly (2 minute intervals for 15 minutes) unstopped (without "stop" buffer). Plates were read at 450nm.

Results

The results of the above experiment are presented in Table 8 and in Figure 5. The animals of group 2 received intranodal injections of ALVAC(2)-gp100(mod) followed by boosts with the modified gp100 peptides 209(2M) and 290(9V); the animals in group 3 received a subcutaneous

injection of the ALVAC(2) construct followed by peptide boosts; the animals in group 1 received intranodal injections of saline as a control.

As can be seen from Figure 5, intranodal injection of the antigens induced a humoral response that was much greater than when the antigen was injected subcutaneously.

In summary, the results of this Example demonstrate that intranodal injection of a tumor antigen induces both a humoral and cell mediated response that is much greater than when the tumor antigen is injected by the conventional subcutaneous route of administration.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

TABLE 1

Group Number	Route of administration	Treatment days and compound administered	Number of Animals
1	Intranodal	Saline (NaCl 0.9%): days 28, 42, 56 Then 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
2	Intranodal	ALVAC(2) - gp100 mod: days 28, 42, 56 *mgp100 peptides: days 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
3	Subcutaneous	Saline (NaCl 0.9%): day 1 ALVAC(2) - gp100 mod: days 28, 42, 56 *mgp100 peptides: days 70 and 84	4

*209(2M)-IMDQVPFSV; 290(9V) YLEPGPVTV

- Group 1 animals (control) received the control article (saline for injection (NaCl 0.9%)).
- Group 3 animals received the control article (saline for injection (NaCl 0.9%)) on day 1 only.

TABLE 2

Group Number	Dose level	Dose volume (ml/administration)
1	Saline (NaCl 0.9%): 0	0.250
2	Dose: 0.25 x $10^{7.4}$ CCID 50 ALVAC (2) - gp100 mod: 0.25 $10^{7.4}$ CCID50	0.250
	Dose: 200 µg (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVTV (290(9V)) (100µg each)	0.2
3	Saline (NaCl 0.9%)	0.250
	ALVAC(2) - gp100 mod: 0.25 $10^{7.4}$ CCID 50	0.250
	Dose: 200 µg (Total) of peptides IMDQVPFSY (209(2M)) and YLEPGPVTV (290(9V)) (100µg each)	0.2

TABLE 3

Days	Sites used
1 and 28	lower left
42	upper left
56	upper right
70	lower left
84	lower right

TABLE 4

Peptide Pool #1

Peptide	Sequence	SEQ.ID.NO.
1329	HLAVIGALLAVGATK	SEQ.ID.NO.3
1330	GALLAVGATKVPRNQ	SEQ.ID.NO.4
1331	VGATKVPRNQDWLGV	SEQ.ID.NO.5
1332	VPRNQDWLGVSRQLR	SEQ.ID.NO.6
1333	DWLGVSRQLRTKAWN	SEQ.ID.NO.7
1334	SRQLRTKAWNRQLYP	SEQ.ID.NO.8
1335	TKAWNRQLYPEWTEA	SEQ.ID.NO.9
1336	RQLYPEWTEAQRLDC	SEQ.ID.NO.10
1337	EWTEAQRLDCWRGGQ	SEQ.ID.NO.11
1338	QRLDCWRGGQVSLKV	SEQ.ID.NO.12
1339	WRGGQVSLKVSNDGP	SEQ.ID.NO.13
1340	VSLKVSNDGPTLIGA	SEQ.ID.NO.14
1344	IALNFPGSQKVLPDG	SEQ.ID.NO.15
1345	PGSQKVLPDGQVIWV	SEQ.ID.NO.16
1346	VLPDGQVIWVNNTII	SEQ.ID.NO.17
1347	QVIWVNNTIINGSQV	SEQ.ID.NO.18
1348	NNTIINGSQVWGGQP	SEQ.ID.NO.19
1349	NGSQVWGGQPVYPQE	SEQ.ID.NO.20
1350	WGGQPVYPQETDDAC	SEQ.ID.NO.21
1351	VYPQETDDACIFPDG	SEQ.ID.NO.22
1352	TDDACIFPDGGPCPS	SEQ.ID.NO.23
1353	IFPDGGPCPSGSWSQ	SEQ.ID.NO.24
1355	GSWSQKRSFVYVWKT	SEQ.ID.NO.25
1356	KRSFVYVWKTWGQYW	SEQ.ID.NO.26
1357	YVWKTWGQYWQVLGG	SEQ.ID.NO.27
1358	WGQYWQVLGGPVSGL	SEQ.ID.NO.28
1359	QVLGGPVSGLSIGTG	SEQ.ID.NO.29

TABLE 5

Peptide Pool #2

Peptide	Sequence	SEQ.ID.NO.
1360	PVSGLSIGTGRAMLG	SEQ.ID.NO.30
1361	SIGTGRAMLGTHTME	SEQ.ID.NO.31
1362	RAMLGTHTMEVTVYH	SEQ.ID.NO.32
1363	THTMEVTVYHRRGSR	SEQ.ID.NO.33
1364	VTVYHRRGSRSYVPL	SEQ.ID.NO.34
1365	RRGSRSYVPLAHSSS	SEQ.ID.NO.35
1366	SYVPLAHSSSAFTIT	SEQ.ID.NO.36
1368	AFTITDQVPFSVSVS	SEQ.ID.NO.37
1369	DQVPFSVSVSQLRAL	SEQ.ID.NO.38
1370	SVSVSQLRALDGGNK	SEQ.ID.NO.39
1372	DGGNKHFLRNQPLTF	SEQ.ID.NO.40
1373	HFLRNQPLTFALQLH	SEQ.ID.NO.41
1374	QPLTFALQLHDPSGY	SEQ.ID.NO.42
1375	ALQLHDPSGYLAEAD	SEQ.ID.NO.43
1379	DFGDSSGTLISRALV	SEQ.ID.NO.44
1380	STGLISRALVVTHTY	SEQ.ID.NO.45
1381	SRALVVTHTYLEPGP	SEQ.ID.NO.46
1382	VTHTYLEPGPVTAQV	SEQ.ID.NO.47
1383	LEPGPVTAQVVLQAA	SEQ.ID.NO.48
1384	VTAQVVLQAAIPLTS	SEQ.ID.NO.49
1385	VLQAAIPLTSCGSSP	SEQ.ID.NO.50
1386	IPLTSCGSSPVPGTT	SEQ.ID.NO.51
1388	VPGTTDGHRPTAEAP	SEQ.ID.NO.52
1389	DGHRPTAEAPNTTAG	SEQ.ID.NO.53
1390	TAEAPNTTAGQVPTT	SEQ.ID.NO.54
1392	QVPTTEVVGTTPGQA	SEQ.ID.NO.55
1393	EVVGTTPGQAPTAEP	SEQ.ID.NO.56

TABLE 6

Peptide Pool #3

Peptide	Sequence	SEQ.ID.NO.
1394	TPGQAPTAEPSGTTS	SEQ.ID.NO.57
1395	PTAEPSGTTSVQVPT	SEQ.ID.NO.58
1396	SGTTSVQVPTTEVIS	SEQ.ID.NO.59
1397	VQVPTTEVISTAPVQ	SEQ.ID.NO.60
1398	TEVISTAPVQMPTAE	SEQ.ID.NO.61
1399	TAPVQMPTAESTGMT	SEQ.ID.NO.62
1400	MPTAESTGMTPEKVP	SEQ.ID.NO.63
1401	STGMTPEKVPVSEVM	SEQ.ID.NO.64
1402	PEKVPVSEVMGTTLA	SEQ.ID.NO.65
1403	VSEVMGTTLAEMSTP	SEQ.ID.NO.66
1404	GTTLAEMSTPEATGM	SEQ.ID.NO.67
1405	EMSTPEATGMTPAEV	SEQ.ID.NO.68
1408	SIVVLSGTTAAQVTT	SEQ.ID.NO.69
1409	SGTTAAQVTTTEWVE	SEQ.ID.NO.70
1410	AQVTTTEWVETTARE	SEQ.ID.NO.71
1411	TEWVETTARELPIPE	SEQ.ID.NO.72
1412	TTARELPIPEPEPGPD	SEQ.ID.NO.73
1413	LPIPEPEGPDASSIM	SEQ.ID.NO.74
1414	PEGPDASSIMSTESI	SEQ.ID.NO.75
1415	ASSIMSTESITGSLG	SEQ.ID.NO.76
1416	STESITGSLGPLLDG	SEQ.ID.NO.77
1417	TGSLGPLLDGTATLR	SEQ.ID.NO.78
1418	PLLDGTATLRLVKRQ	SEQ.ID.NO.79
1419	TATLRLVKRQVPLDC	SEQ.ID.NO.80
1420	LVKRQVPLDCVLYRY	SEQ.ID.NO.81
1421	VPLDCVLYRYGSFSV	SEQ.ID.NO.82
1422	VLYRYGSFSVTLDIV	SEQ.ID.NO.83

Table 7

Peptide Pool #4

Peptide	Sequence	SEQ.ID.NO
1424	TLDIVQGIESAEILQ	SEQ.ID.NO.84
1425	QGIESAEILQAVPSG	SEQ.ID.NO.85
1426	AEILQAVPSGEGDAF	SEQ.ID.NO.86
1427	AVPSGEGDAFELTVS	SEQ.ID.NO.87
1428	EGDAFELTVSCQGGL	SEQ.ID.NO.88
1429	ELTVSCQGGLPKEAC	SEQ.ID.NO.89
1430	CQGGLPKEACMEISS	SEQ.ID.NO.90
1431	PKEACMEISSPGCQP	SEQ.ID.NO.91
1432	MEISSPGCQPPAQRL	SEQ.ID.NO.92
1434	PAQRLCQPVLPSPAC	SEQ.ID.NO.93
1435	CQPVLPSPACQLVLH	SEQ.ID.NO.94
1436	PSPACQLVLHQILKG	SEQ.ID.NO.95
1437	QLVLHQILKGGSGTY	SEQ.ID.NO.96
1441	LADTNSLAVVSTQLI	SEQ.ID.NO.97
1442	SLAVVSTQLIMPGQE	SEQ.ID.NO.98
1443	STQLIMPGQEAGLGQ	SEQ.ID.NO.99
1444	MPGQEAGLGQVPLIV	SEQ.ID.NO.100
1445	AGLGQVPLIVGILLV	SEQ.ID.NO.101
1448	LMAVVLASLIYRRRL	SEQ.ID.NO.102
1450	YRRRLMKQDFSVPQL	SEQ.ID.NO.103
1451	MKQDFSVPQLPHSSS	SEQ.ID.NO.104
1452	SVPQLPHSSSHWLRL	SEQ.ID.NO.105
1453	PHSSSHWLRLPRIFC	SEQ.ID.NO.106
1454	HWLRLPRIFCSCPIG	SEQ.ID.NO.107
1455	PRIFCSCPIGENSPL	SEQ.ID.NO.108

TABLE 8

	DAY (mOD/min)			
Monkey #	0	57	68	96
1	3	5	2	2
2	4	6	12	10
3	7	6	10	8
4	7	6	8	8
5	5	9	20	15
6	11	8	10	12
7	11	23	51	30
8	7	30	70	22
9	1	7	5	3
10	2	6	6	4
11	3	7	14	8
12	6	9	15	6

We claim:

1. A method for inducing an immune response in an animal to a tumor antigen comprising administering an effective amount of a tumor antigen or a nucleic acid sequence encoding a tumor antigen to a lymphatic site in the animal.

2. A method according to claim 1 wherein the tumor antigen is selected from the group consisting of CEA, gp100, the MAGE family of proteins, DAGE, GAGE, RAGE, NY-ESO 1, Melan-A/MART 1, TRP-1, TRP-2, tyrosinase, HER-2/neu, MUC-1, p53, KSA, PSA, PSMA, and fragments and modified versions thereof.

3. A method according to claim 1 or 2 wherein the lymphatic site is a lymph node.

4. A method according to any one of claims 1 to 3 wherein the nucleic acid is selected from the group consisting of viral nucleic acid, bacterial DNA, plasmid DNA, naked/free DNA, and RNA.

5. A method according to claim 4 wherein the viral nucleic acid is selected from the group consisting of adenoviral, alphaviral and poxviral nucleic acid.

6. A method according to claim 5 wherein the poxviral nucleic acid is selected from the group consisting of avipox, orthopox and suipox nucleic acid.

7. A method according to claim 5 wherein the poxviral nucleic acid is selected from the group consisting of vaccinia, fowl pox, canarypox and swinepox nucleic acid.

8. A method according to claim 5 wherein the poxviral nucleic acid is selected from the group consisting of MVA, NYVAC, TROVAC, and ALVAC nucleic acid.

9. A method according to any one of claims 1 to 8 wherein the nucleic acid is contained in a vector.

10. A method according to claim 9 wherein the vector is a recombinant virus or bacteria.

11. A method according to claim 10 wherein the recombinant virus is selected from the group consisting of adenovirus, alphavirus and poxvirus.

12. A method according to claim 11 wherein the poxvirus is selected from the group consisting of avipox, orthopox and suipox.

13. A method according to claim 11 wherein the poxvirus is selected from the group consisting of vaccinia, fowlpox, canarypox and swinepox.

14. A method according to claim 11 wherein the poxvirus is selected from the group consisting of MVA, NYVAC, TROVAC, and ALVAC.

15. A method according to any one of claims 1 to 8 wherein the nucleic acid is contained in a cell.

16. A method according to any one of claims 1 to 14 wherein the tumor antigen or nucleic acid coding therefor is contained in a vaccine.

17. A method according to any one of claims 1 to 16 wherein the tumor antigen is gp100, CEA or a fragment or modified version of gp100 or CEA.

18. A method according to claim 17 wherein the modified gp100 comprises the sequence IMDQVPFSY (SEQ ID NO: 1) and/or YLEPGPVTV (SEQ ID NO:2).

19. A method according to claim 17 wherein the modified CEA comprises the sequence shown in Figure 8 (SEQ ID NO:112) and/or YLSGADLNL (SEQ ID NO:113).

FIGURE 1

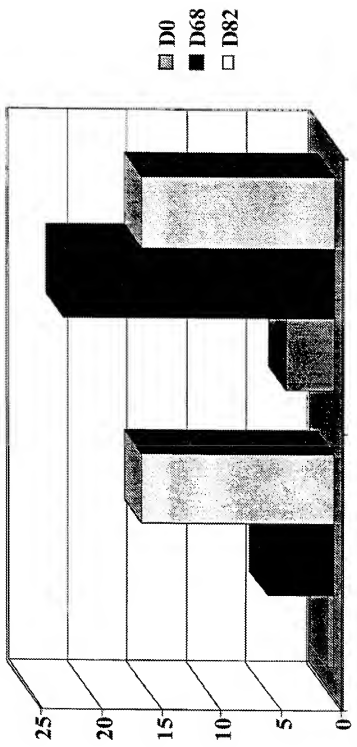

Monkey #6 (Intranodal Administration)
D0
D68
D82
Spots/1E6 Cells
0
5
10
15
20
25
Pool 3
Pool4

FIGURE 2

Monkey #7 (Intranodal Administration)

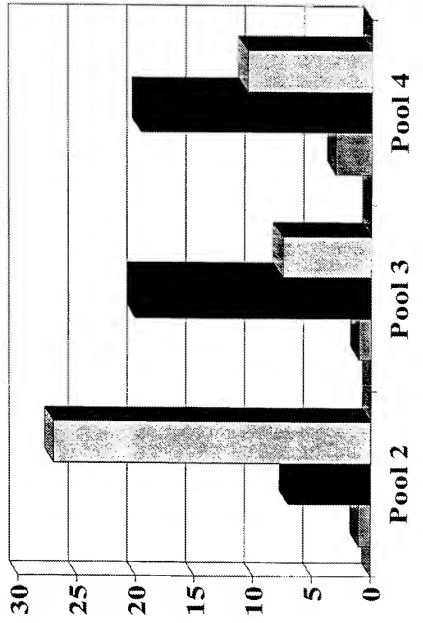


FIGURE 3

Monkey # 11 (Subcutaneous Administration)

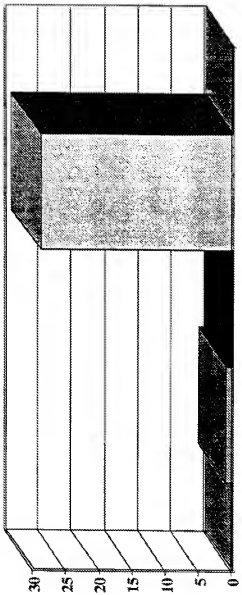


FIGURE 4

Monkey #10 (Subcutaneous Administration)

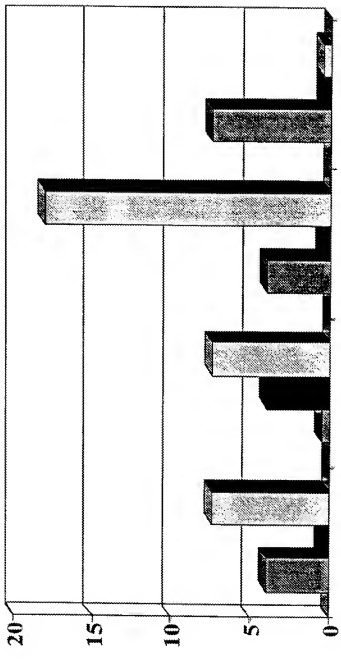
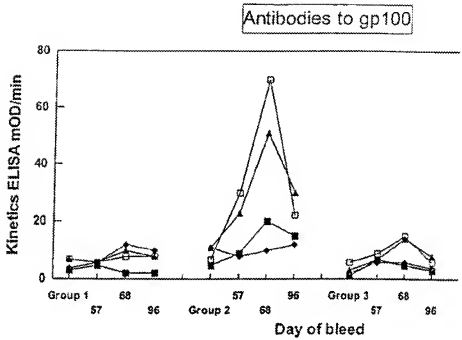


FIGURE 5


Antibodies to gp100
Kinetics ELISA mOD/min
80
60
40
20
0
Group 1
57
68
96
Group 2
57
68
96
Group 3
57
68
96
Day of bleed

6/11

FIGURE 6

```
                ATGG ATCTGGTGCT AAAAAGATGC CTTCTTCATT TGGCTGTGAT
AGGTGCTTTG CTGGCTGTGG GGGCTACAAA AGTACCCAGA AACCAGGACT GGCTTGGTGT
CTCAAGGCAA CTCAGAACCA AAGCCTGGAA CAGGCAGCTG TATCCAGAGT GGACAGAAGC
CCAGAGACTT GACTGCTGGA GAGGTGGTCA AGTGTCCCTC AAGGTCAGTA ATGATGGGCC
TACACTGATT GGTGCAAATG CCTCCTTCTC TATTGCCTTG AACTTCCCTG GAAGCCAAAA
GGTATTGCCA GATGGGCAGG TTATCTGGGT CAACAATACC ATCATCAATG GGAGCCAGGT
GTGGGGAGGA CAGCCAGTGT ATCCCCAGGA AACTGACGAT GCCTGCATCT TCCCTGATGG
TGGACCTTGC CCATCTGGCT CTTGGTCTCA GAAGAGAAGC TTTGTTTATG TCTGGAAGAC
CTGGGGCCAA TACTGGCAAG TTCTAGGGGG CCCAGTGTCT GGGCTGAGCA TTGGGACAGG
CAGGGCAATG CTGGGCACAC ACACGATGGA AGTGACTGTC TACCATCGCC GGGGATCCCG
GAGCTATGTG CCTCTTGCTC ATTCCAGCTC AGCCTTCACC ATTATGGACC AGGTGCCTTT
CTCCGTGAGC GTGTCCAGT TGCGGGCCTT GGATGGAGGG AACAAGCACT TCCTGAGAAA
TCAGCCTCTG ACCTTTGCCC TCCAGCTCCA TGACCCCAGT GGCTATCTGG CTGAAGCTGA
CCTCTCCTAC ACCTGGGACT TTGGAGACAG TAGTGGAACC CTGATCTCTC GGGCACTTGT
GGTCACTCAT ACTTACCTGG AGCCTGGCCC AGTCACTGTT CAGGTGGTCC TGCAGGCTGC
CATTCCTCTC ACCTCCTGTG GCTCCTCCCC AGTTCCAGGC ACCACAGATG GGCACAGGCC
AACTGCAGAG GCCCCTAACA CCACAGCTGG CCAAGTGCCT ACTACAGAAG TTGTGGGTAC
TACACCTGGT CAGGCGCCAA CTGCAGAGCC CTCTGGAACC ACATCTGTGC AGGTGCCAAC
CACTGAAGTC ATAAGCACTG CACCTGTGCA GATGCCAACT GCAGAGAGCA CAGGTATGAC
ACCTGAGAAG GTGCCAGTTT CAGAGGTCAT GGGTACCACA CTGGCAGAGA TGTCAACTCC
AGAGGCTACA GGTATGACAC CTGCAGAGGT ATCAATTGTG GTGCTTTCTG GAACCACAGC
TGCACAGGTA ACAACTACAG AGTGGGTGGA GACCACAGCT AGAGAGCTAC CTATCCCTGA
GCCTGAAGGT CCAGATGCCA GCTCAATCAT GTCTACGGAA AGTATTACAG GTTCCCTGGG
CCCCCTGCTG GATGGTACAG CCACCTTAAG GCTGGTGAAG AGACAAGTCC CCCTGGATTG
TGTTCTGTAT CGATATGGTT CCTTTTCCGT CACCCTGGAC ATTGTCCAGG GTATTGAAAG
TGCCGAGATC CTGCAGGCTG TGCCGTCCGG TGAGGGGGAT GCATTTGAGC TGACTGTGTC
CTGCCAAGGC GGGCTGCCCA AGGAAGCCTG CATGGAGATC TCATCGCCAG GGTGCCAGCC
CCCTGCCCAG CGGCTGTGCC AGCCTGTGCT ACCCAGCCCA GCCTGCCAGC TGGTTCTGCA
CCAGATACTG AAGGGTGGCT CGGGGACATA CTGCCTCAAT GTGTCTCTGG CTGATACCAA
CAGCCTGGCA GTGGTCAGCA CCCAGCTTAT CATGCCTGGT CAAGAAGCAG GCCTTGGGCA
GGTTCCGCTG ATCGTGGGCA TCTTGCTGGT GTTGATGGCT GTGGTCCTTG CATCTCTGAT
ATATAGGCGC AGACTTATGA AGCAAGACTT CTCCGTACCC CAGTTGCCAC ATAGCAGCAG
TCACTGGCTG CGTCTACCCC GCATCTTCTG CTCTTGTCCC ATTGGTGAGA ACAGCCCCCT
CCTCAGTGGG CAGCAGGTCT GA
```

FIGURE 7

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Met Asp Leu Val Leu Lys Arg Cys Leu Leu His Leu Ala Val Ile Gly
  1               5                  10                  15
Ala Leu Leu Ala Val Gly Ala Thr Lys Val Pro Arg Asn Gln Asp Trp
            20                  25                  30
Leu Gly Val Ser Arg Gln Leu Arg Thr Lys Ala Trp Asn Arg Gln Leu
        35                  40                  45
Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
    50                  55                  60
Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
65                  70                  75                  80
Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
                85                  90                  95
Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
            100                 105                 110
Ser Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Thr Asp Asp
        115                 120                 125
Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser
    130                 135                 140
Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
145                 150                 155                 160
Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
                165                 170                 175
Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
            180                 185                 190
Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Glu Ala Phe Thr
        195                 200                 205
Ile Met Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
    210                 215                 220
Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
225                 230                 235                 240
Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
                245                 250                 255
Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
            260                 265                 270
Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Val
        275                 280                 285
Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
    290                 295                 300
Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
305                 310                 315                 320
Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
                325                 330                 335
Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
            340                 345                 350
Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr
        355                 360                 365
```

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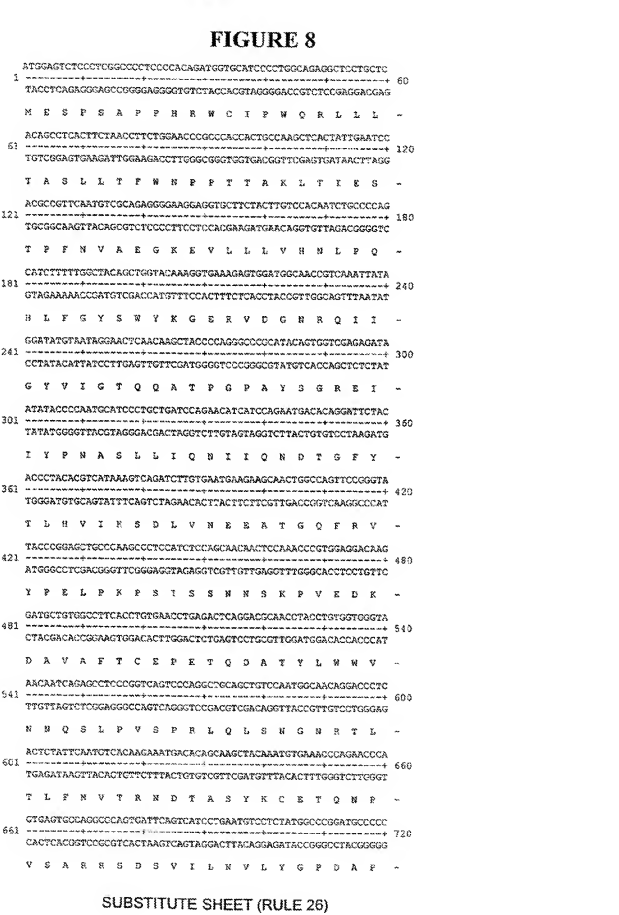
FIGURE 7 (CONT'D)

```
Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val
    370                 375                 380
Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met
385                 390                 395                 400
Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
                405                 410                 415
Gln Val Thr Thr Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu Pro
            420                 425                 430
Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu
        435                 440                 445
Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu
    450                 455                 460
Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr
465                 470                 475                 480
Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala
                485                 490                 495
Glu Ile Leu Gln Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu Leu
            500                 505                 510
Thr Val Ser Cys Gln Gly Gly Leu Pro Lys Glu Ala Cys Met Glu Ile
        515                 520                 525
Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val
    530                 535                 540
Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly
545                 550                 555                 560
Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
                565                 570                 575
Leu Ala Val Val Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala Gly
            580                 585                 590
Leu Gly Gln Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu Met Ala
        595                 600                 605
Val Val Leu Ala Ser Leu Ile Tyr Arg Arg Arg Leu Met Lys Gln Asp
    610                 615                 620
Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu
625                 630                 635                 640
Pro Arg Ile Phe Cys Ser Cys Pro Ile Gly Glu Asn Ser Pro Leu Leu
                645                 650                 655
Ser Gly Gln Gln Val
            660
```

FIGURE 8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1
60
TACCTCAGAGGGAGCCGGGGAGGGGTGTCTACCACGTAGGGGACCGTCTCCGAGGACGAG
a
M E S P S A P F H R W C I P W Q R L L L -
ACAGCCTCACTTCTAACCTTCTGGAACCCGCCCACCACTGCCAAGCTCACTATTGAATCC
61
120
TGTCGGAGTGAAGATTGGAAGACCTTGGGCGGGTGGTGACGGTTCGAGTGATAACTTAGG
a
T A S L L T F W N P P T T A K L T I E S -
ACGCCGTTCAATGTCGCAGAGGGGAAGGAGGTGCTTCTACTTGTCCACAATCTGCCCCAG
121
180
TGCGGCAAGTTACAGCGTCTCCCCTTCCTCCAGGAAGATGAACAGGTGTTAGACGGGGTC
a
T P F N V A E G K E V L L L V H N L P Q -
CATCTTTTTGGCTACAGCTGGTACAAAGGTGAAAGAGTGGATGGCAACCGTCAAATTATA
181
240
GTAGAAAAACCGATGTCGACCATGTTTCCACTTTCTCACCTACCGTTGGCAGTTTAATAT
a
H L F G Y S W Y K G E R V D G N R Q I I -
GGATATGTAATAGGAACTCAACAAGCTACCCCAGGGCCCCCATACAGTGGTCGAGAGATA
241
300
CCTATACATTATCCTTGAGTTGTTCGATGGGGTCCCGGGCGTATGTCACCAGCTCTCTAT
a
G Y V I G T Q Q A T P G P A Y S G R E I -
ATATACCCCAATGCATCCCTGCTGATCCAGAACATCATCCAGAATGACACAGGATTCTAC
301
360
TATATGGGGTTACGTAGGGACGACTAGGTCTTGTAGTAGGTCTTACTGTGTCCTAAGATG
a
I Y P N A S L L I Q N I I Q N D T G F Y -
ACCCTACACGTCATAAAGTCAGATCTTGTGAATGAAGHAGCAACTGGCCAGTTCCGGGTA
361
420
TGGGATGTGCAGTATTTCAGTCTAGAACACTTACTTCTTCGTTGACCGGTCAAGGCCCAT
a
T L H V I F S D L V N E E A T G Q F R V -
TACCGGAGCTGCCCAAGCCCTCCATCTCCAGCAACAACTCCAAACCCGTGGAGGACAAG
421
480
ATGGCCTCGACGGGTTCGGGAGGTAGAGGTCGTTGTTGAGGTTTGGGCACCTCCTGTTC
a
Y P E L P K P S I S S N N S K P V E D K -
GATGCTGTGGCCTTCACCTGTGAACCTGAGACTCAGGACGCAACCTACCTGTGGTGGGTA
481
540
CTACGACACCGCAAGTGGACACTTGGACTCTGAGTCCTGCGTTGGATGGACACCACCCAT
a
D A V A F T C E P E T Q D A T Y L W W V -
AACAATCAGAGCCTCCCGGTCAGTCCCAGGCCTCGCAGCTGTCCAATGGCAACAGGACCCTC
541
600
TTGTTAGTCTCGGAGGGCCAGTCAGGGTCCGACGTCGACAGGTTACCGTTGTCCTGGGAG
a
N N Q S L P V S P R L Q L S N G N R T L -
ACTCTATTCAATGTCACAAGAAATGACACAGCAAGCTACAAATGTGAAACCCAGAACCCA
601
660
TGAGATAAGTTACACTCTTCTTTACTGTGTCGTTCGATGTTTACACTTTGGGTCTTGGGT
a
T L F N V T R N D T A S Y K C E T Q N P -
GTGAGTGCCAGGCCCAGCGATTCAGTCATCCTGAATGTCCTCTATGGCCCGGATGCCCCC
661
720
CACTCACGGTCCGCGTCACTAAGTCAGTAGGACTTACAGGAGATACCGGGCCTACGGGGG
a
V S A R S S D S V I L N V L Y G P D A P -

FIGURE 8 (CONT'D)

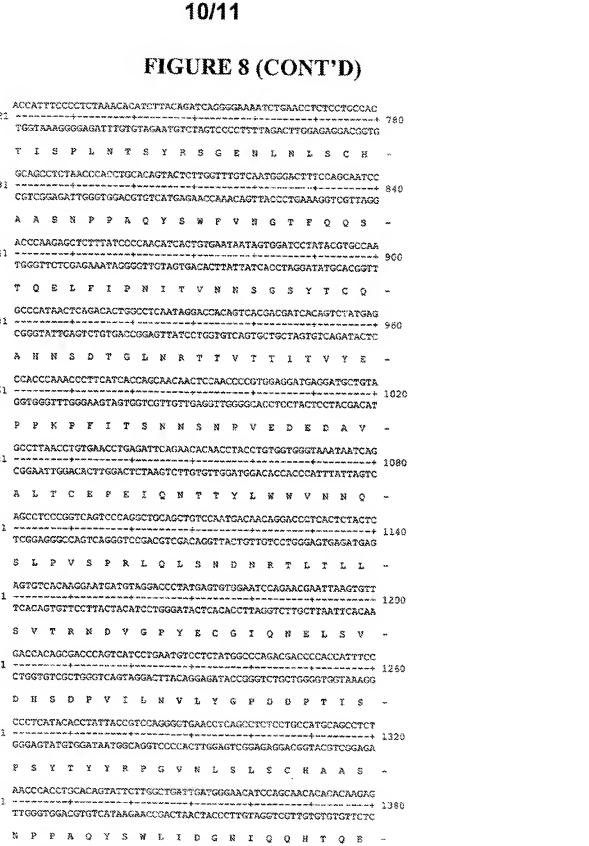
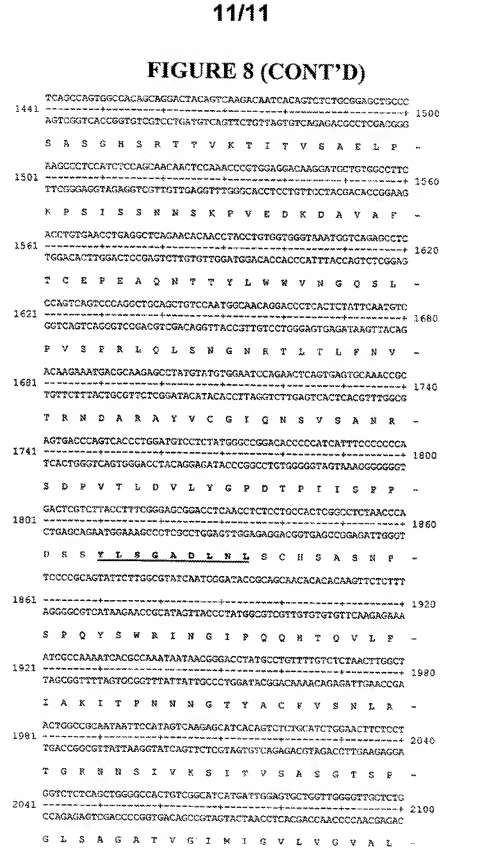


FIGURE 8 (CONT'D)



INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/CA 00/01253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/00 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, CANCERLIT, LIFESCIENCES, EMBASE, SCISEARCH, EPO-Internal, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 47271 A (GUO YAJUN) 18 December 1997 (1997-12-18) page 23, line 14 -page 24, line 22	1-3,15, 16
X	RAO V S ET AL: "PARTIAL CHARACTERIZATION OF TWO SUBPOPULATIONS OF T-4 CELLS INDUCED BY ACTIVE SPECIFIC INTRALYMPHATIC IMMUNOTHERAPY IN MELANOMA PATIENTS" PROCEEDINGS AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 27, 1986, page 325 XP000990377 ISSN: 0197-016X the whole document	1,2,16

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[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 March 2001	26/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

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INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/CA 00/01253

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MACKENSEN ANDREAS ET AL: "Homing of intravenously and intralymphatically injected human dendritic cells generated in vitro from CD34+ hematopoietic progenitor cells." CANCER IMMUNOLOGY IMMUNOTHERAPY, vol. 48, no. 2-3, May 1999 (1999-05), pages 118-122, XP000990346 ISSN: 0340-7004 the whole document ---	1-19
A	IRVINE KARI R ET AL: "Recombinant virus vaccination against "self" antigens using anchor-fixed immunogens." CANCER RESEARCH, vol. 59, no. 11, 1 June 1999 (1999-06-01), pages 2536-2540, XP002161590 ISSN: 0008-5472 the whole document -----	1-19

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Information on patent family members

International Application No
PCT/CA 00/01253

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		CA 2258082 A	18-12-1997
		CN 1221349 A	30-06-1999
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